Whole Effluent Toxicity (WET)

Staff Training

August 29, 2012

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Species and Biological Endpoints

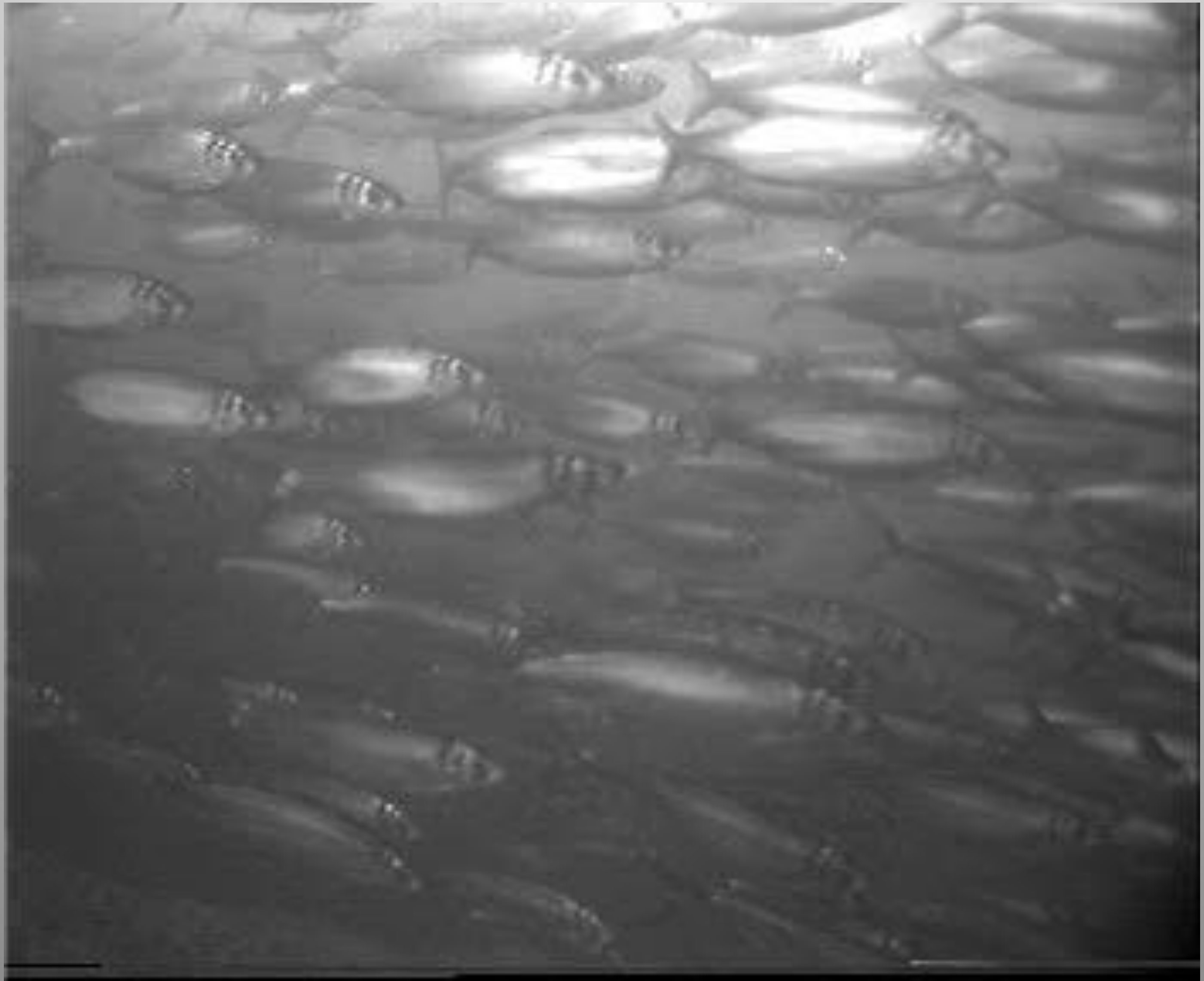
- Acute tests look at 96-hour survival (fish) or 48-hour survival (daphnids and mysids).
- 7-day survival and growth tests with fish or mysids measure survival, biomass, and weight.
- The *Ceriodaphnia* chronic test assesses 7-day survival and asexual reproduction (neonates).
- Bivalve (oysters or mussels) and echinoderm (urchins or sand dollars) tests measure survival and normal development after a couple days.
- Echinoderms are also used in a 40-minute chronic test assessing fertilization.
- EPA defines a chronic test not by duration but by having a sublethal (weight, fertilization, etc.) endpoint.

fathead minnow (*Pimephales promelas*)

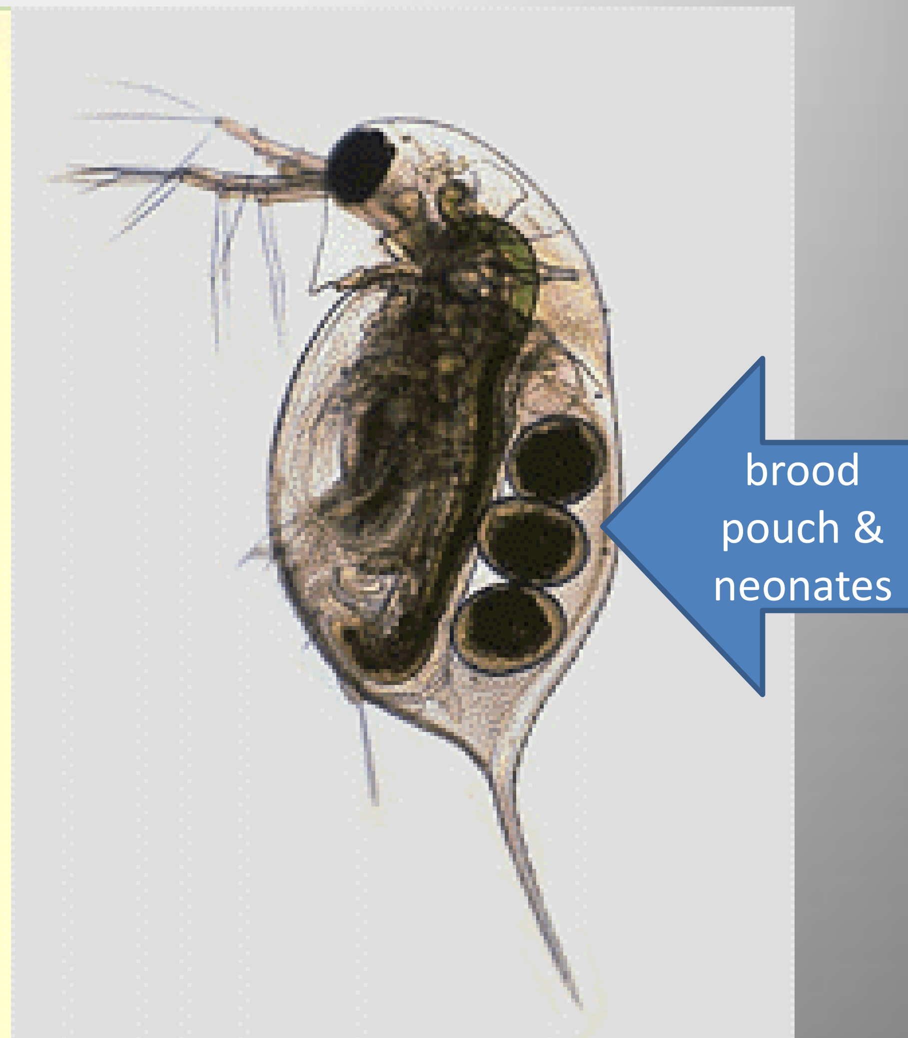
representative salmonid (*Oncorhynchus clarkii*)

topsmelt (*Atherinops affinis*)

Pacific herring (*Clupea pallasii*)

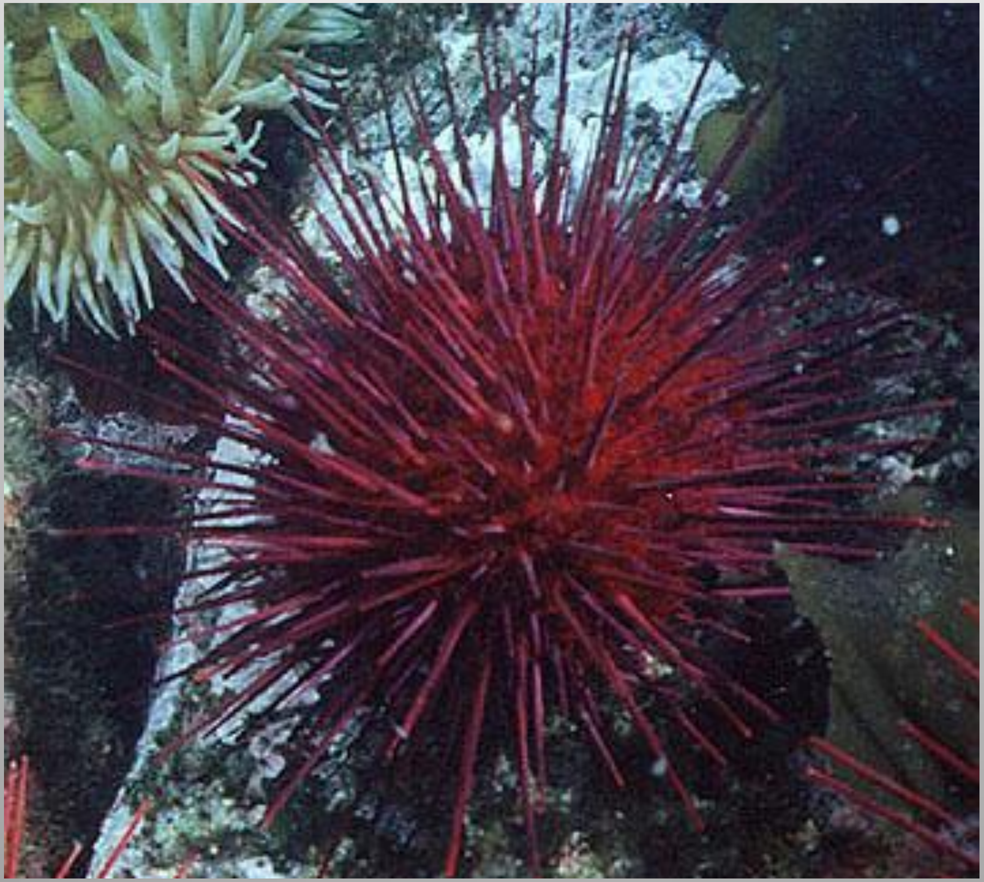


Daphnia magna *Daphnia pulex*



a mysid similar to *Americamysis bahia*

purple sea urchin (*Strongylocentrotus purpuratus*)



diseased *Ceriodaphnia*

(representative of potential test interference by pathogens)

example of test conditions from Canary Book (link on last slide)

Fathead Minnow Survival and Growth

- Test species: *Pimephales promelas*
- Approved test method: EPA-821-R-02-013, method 1000.0
- Test type: 7-day static-renewal (80% renewal of test solution in each test chamber daily)
- Temperature: 25° ± 1°C
- Illumination: Illumination for 16 hours at 10 - 20 µE/m^2/s (50 - 100 ft-c) followed by 8 hours of darkness.
- Test chamber size: 500 mL (minimum)
- Test solution volume: 250 mL (minimum)
- Age of test organisms: < 24 hours (< 48 hours if shipped)
- Number of organisms/chamber: 10
- Number of replicates/concentration: 4 (minimum)
- Feeding: 0.1 g wet weight (approximately 1,000 *Artemia* nauplii) per container 3 times daily at 4-hour intervals (4 times/day at 2.5- to 3.0-hour intervals is acceptable) or 0.15 g wet weight (approximately 1,500 *Artemia* nauplii) per container twice daily at 6 hour intervals: no food in final 12 hours
- Aeration: none unless DO < 4.0 mg/L; aerate all chambers and use < 100 bubbles/minute
- Test duration: 7 days
- Endpoints: survival rate
- total weight of survivors divided by the initial count (**biomass**)
- total weight of survivors divided by the final count (**weight**)
- Control performance criteria: ≥ 80% survival in the control
- average dry weight ≥ 0.25 mg per surviving fish in the control
- Data entry: Because biomass can be zero, total weight equals tare weight for each replicate with zero survival. Because division by zero is undefined, the pan count should be blank for each replicate with zero survival. See **Appendix C.** for more explanation.

test chambers in racks

typical survival & growth datasheet

Conc-%	Code	Rep	Pos	start	1 day	2 day	3 day	4 day	5 day	6 day	7 day	Total mg	Tare mg	Pan Count
0	D	1	19	10	10	10	10	10	10	10	10	96.25	88.03	10
0	D	2	8	10	10	10	10	10	10	10	10	91.65	82.65	10
0	D	3	2	10	10	10	10	10	10	10	10	92.48	84.93	10
0	D	4	23	10	10	10	10	10	10	10	10	95.33	86.66	10
8.8		1	11	10	10	10	10	10	10	10	10	92.22	84.44	10
8.8		2	5	10	9	9	9	9	9	9	9	94.8	87.54	9
8.8		3	14	10	9	9	9	9	9	9	9	94.72	88.06	9
8.8		4	7	10	10	10	10	10	10	10	10	91.22	84.07	10
12.5		1	3	10	10	10	10	10	10	10	10	98.35	90.89	10
12.5		2	20	10	10	10	10	10	10	10	10	102.14	92.65	10
12.5		3	22	10	10	10	10	10	10	10	10	101.44	93.56	10
12.5		4	12	10	10	10	10	10	10	10	10	95.06	86.51	10
25		1	6	10	10	10	10	10	10	10	10	95.44	87.04	10
25		2	13	11	11	11	11	11	11	11	11	91.6	84.02	11
25		3	16	10	10	10	10	10	10	10	10	98.62	89.17	10
25		4	18	10	10	10	10	10	10	10	10	94.13	86.24	10
50		1	15	10	10	10	10	10	10	10	10	96.04	88.29	10
50		2	17	10	10	10	10	10	10	10	10	94.26	86.05	10
50		3	21	10	10	10	10	10	10	10	10	94.14	85.39	10
50		4	4	10	10	10	10	10	10	10	10	93.95	86.29	10
100		1	9	10	10	10	10	10	10	10	10	97.5	89.04	10
100		2	1	10	10	10	10	10	10	10	10	95.83	87.92	10
100		3	24	10	10	10	10	10	10	10	10	97.51	88.76	10
100		4	10	10	10	10	10	10	10	10	10	96.42	88.16	10

Biological Endpoint Calculations

- survival = # final ÷ # start
- biomass = (total weight – tare) ÷ # start
- weight = (total weight – tare) ÷ pan count

The Canary Book (link on last slide) shows the formulas for the other endpoint calculations such as reproduction, development, and fertilization.

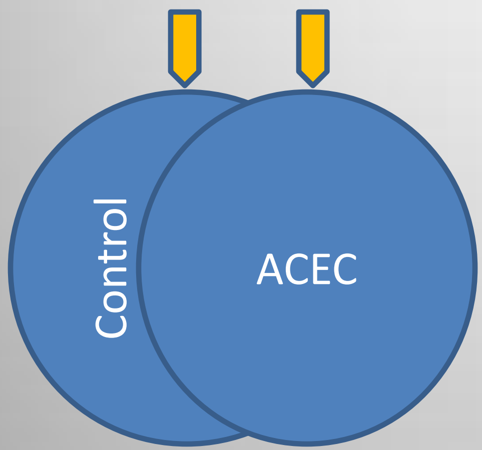
Statistics mean never having to say you're certain.

The official name for the average is the mean. Because the term "mean" is unavoidable in CETIS printouts and statistics discussions, I use it here in this presentation.

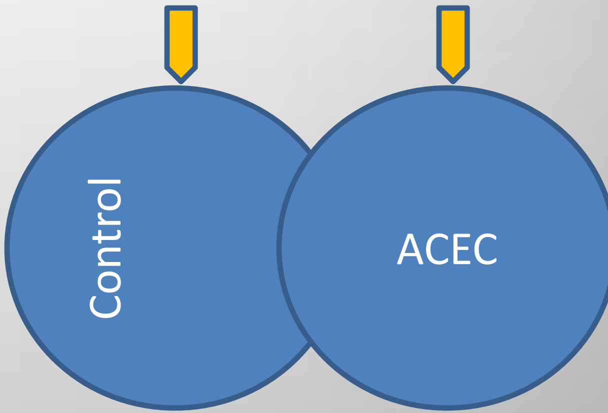
ACEC or CCEC versus control comparison – statistically significant or not?

- The answer is determined by
  - assuming that the numbers in the ACEC (or CCEC) and in the control are from the same population (null hypothesis = no difference),
  - calculating the probability that any differences seen between the ACEC and the control are due to chance sampling of the same population, and
  - rejecting the null hypothesis and assuming that differences indicate toxicity if this probability is low ($p < 0.05$ or $p < 0.01$).

one population or two?



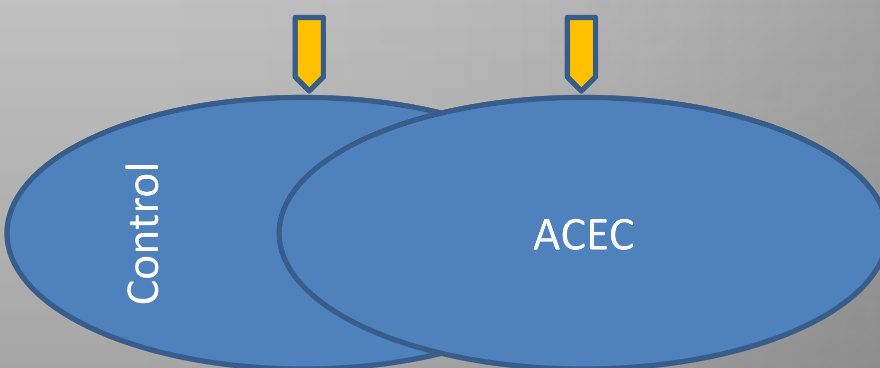
Orange arrows point to the middle of circles to represent population means.



Not significant and easy to imagine the two circles as really being one.

Clearly two separate circles and enough distance between arrows to be significant.

Same distance between arrows as above on right, but the spread of the circles (variability and overlap) makes it not significant.



Definitions

- LOEC (lowest observed effects concentration) = lowest test concentration with a statistically significant difference from the control.
- NOEC (no observed effects concentration) = highest test concentration with no statistically significant difference from the control (or simply the concentration just below the LOEC).
- PMSD (percent minimum significant difference) = the smallest difference which would be statistically significant expressed as the percent difference from control response.
- For example, a PMSD of 15% means that a 15% reduction in survival, etc. from the control would be significant but a 14% reduction would not.

Purposes

- If the LOEC ≤ the ACEC (or CCEC), then the discharge either needs a chronic WET limit or failed to meet a WET limit (acute or chronic).
- If the NOEC ≥ the ACEC (or CCEC), the discharge passed at levels of regulatory concern.
- Close results need to be reanalyzed as single comparisons as per WAC 173-205-070(1) & (2).
- The PMSD for acute tests must be ≤ 30% and the PMSD for chronic tests must be ≤ 40%.

Point Estimates (LC_{50}, IC_{25}, EC_{25}) - benefits

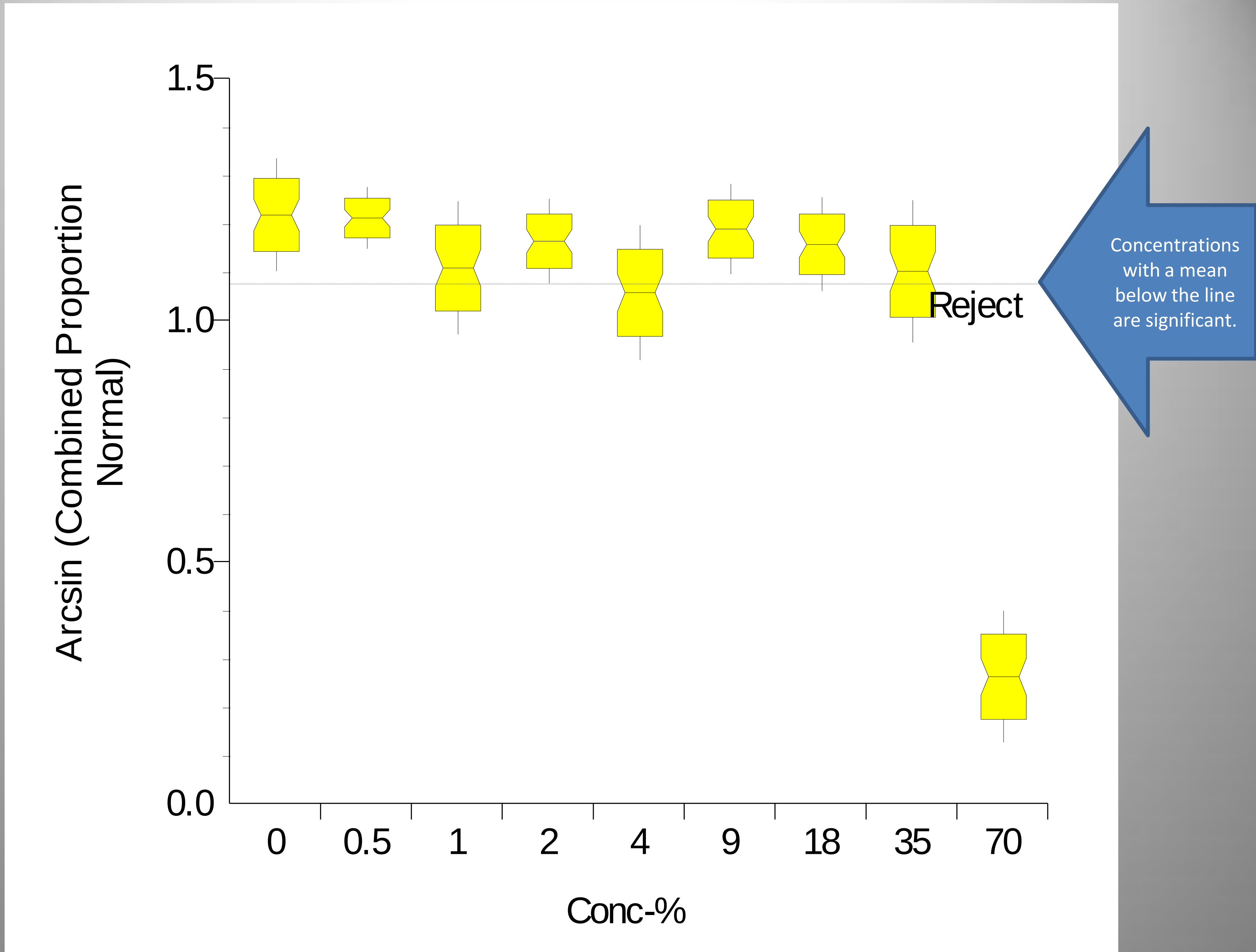
- Point estimates can be found anywhere within the range of concentrations tested and are not confined only to the test concentrations themselves as are NOECs and LOECs.
- Because point estimates occur anywhere in a concentrations series, they are good for comparing toxicity between tests:
  – Is substance X more toxic than substance Y?
  – Has discharge toxicity increased since last year?

Point Estimates - downside

- Regulatory use of point estimates requires what is usually a subjective choice of an effect level (50%, 25%, etc.) which will not necessarily equal the degree of effect seen in tests or the environment.
- Point estimates have confidence intervals that extend in both directions and must be ignored unless you are willing to choose a direction in which to err.
- Point estimates are derived using only the mean responses and do not account for the variability of the measurements used in calculating those means.

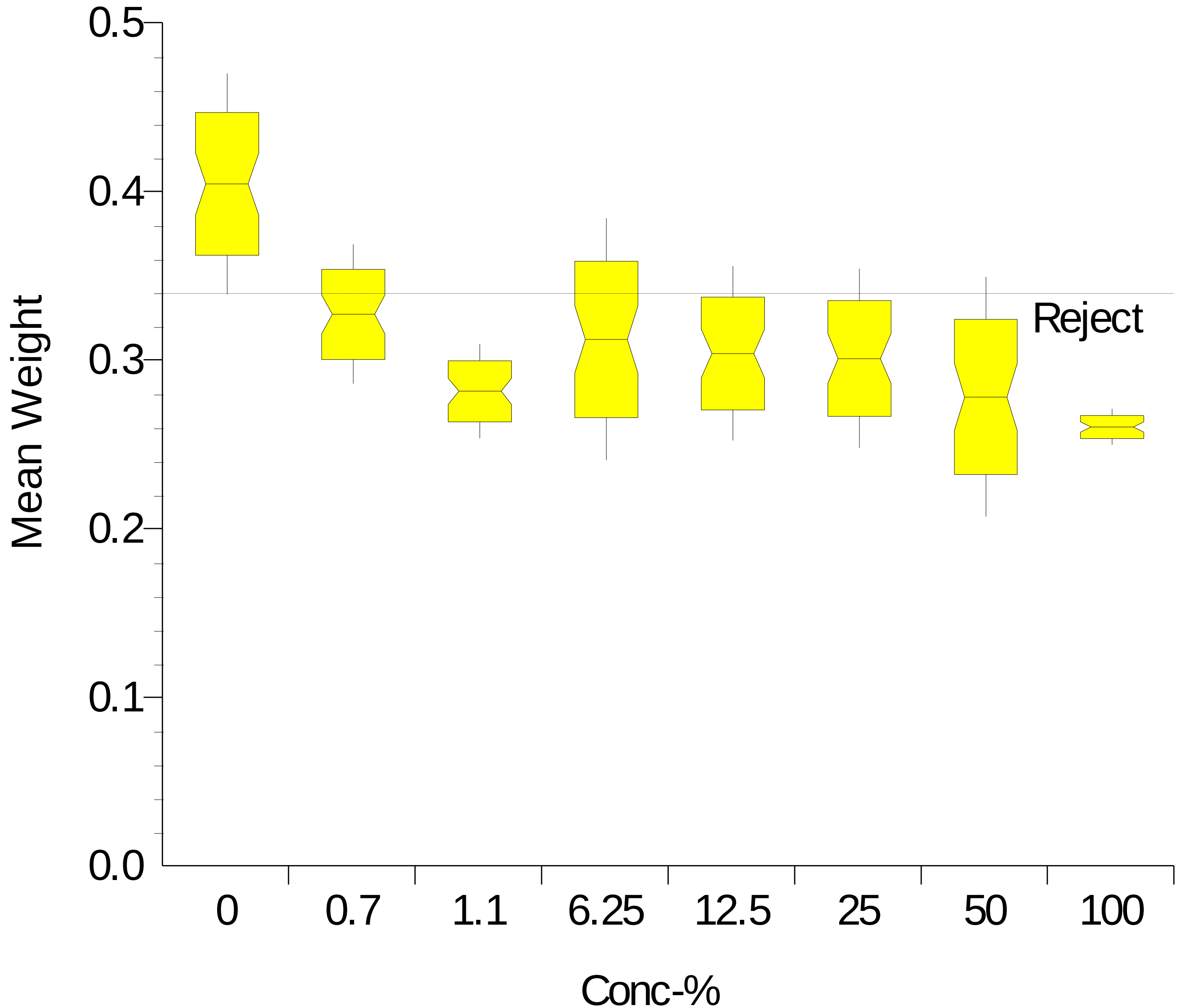
Anomalous test result criterion 1

A WET test result is anomalous if it shows a statistically significant difference in response between the control and the ACEC or CCEC, but no statistically significant difference at one or more higher effluent concentrations.


1.5
1.0
0.5
0.0
Arcsin (Combined Proportion Normal)
0
0.5
1
2
4
9
18
35
70
Conc-%
Reject
Concentrations with a mean below the line are significant.

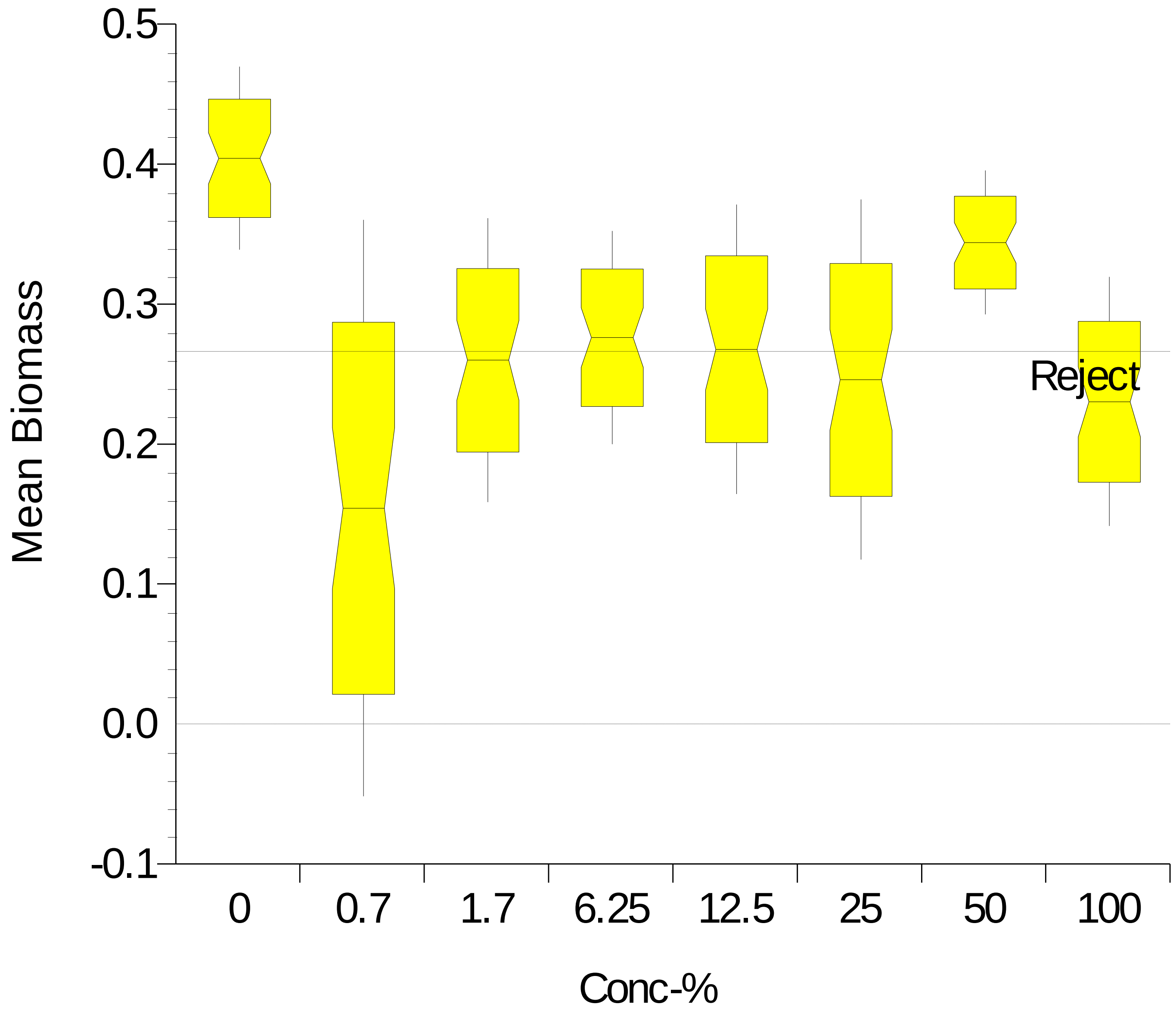
Anomalous test result criterion 2

- A WET test is anomalous if there is a statistically significant difference in response between the control and the ACEC or CCEC which together with nearby concentrations have a zero slope and appear to be nontoxic (performance is typical of healthy test organisms).
- Another description of this criterion is a test with a control that seems to not belong to the concentration-response relationship because of exceptionally good performance.


Mean Weight
0.5
0.4
0.3
0.2
0.1
0.0
Reject
0
0.7
1.1
6.25
12.5
25
50
100
Conc-%

Anomalous test result criterion 3

- A WET test is anomalous if the standard deviation for proportion alive equals or exceeds 0.3 unless the partial mortality fits a good concentration-response relationship.
- The following example meets all three anomalous criteria and the biomass results are driven by highly variable survival.
- Pathogen perhaps? (Lab technician notations on the lab bench sheet may have the answer.)

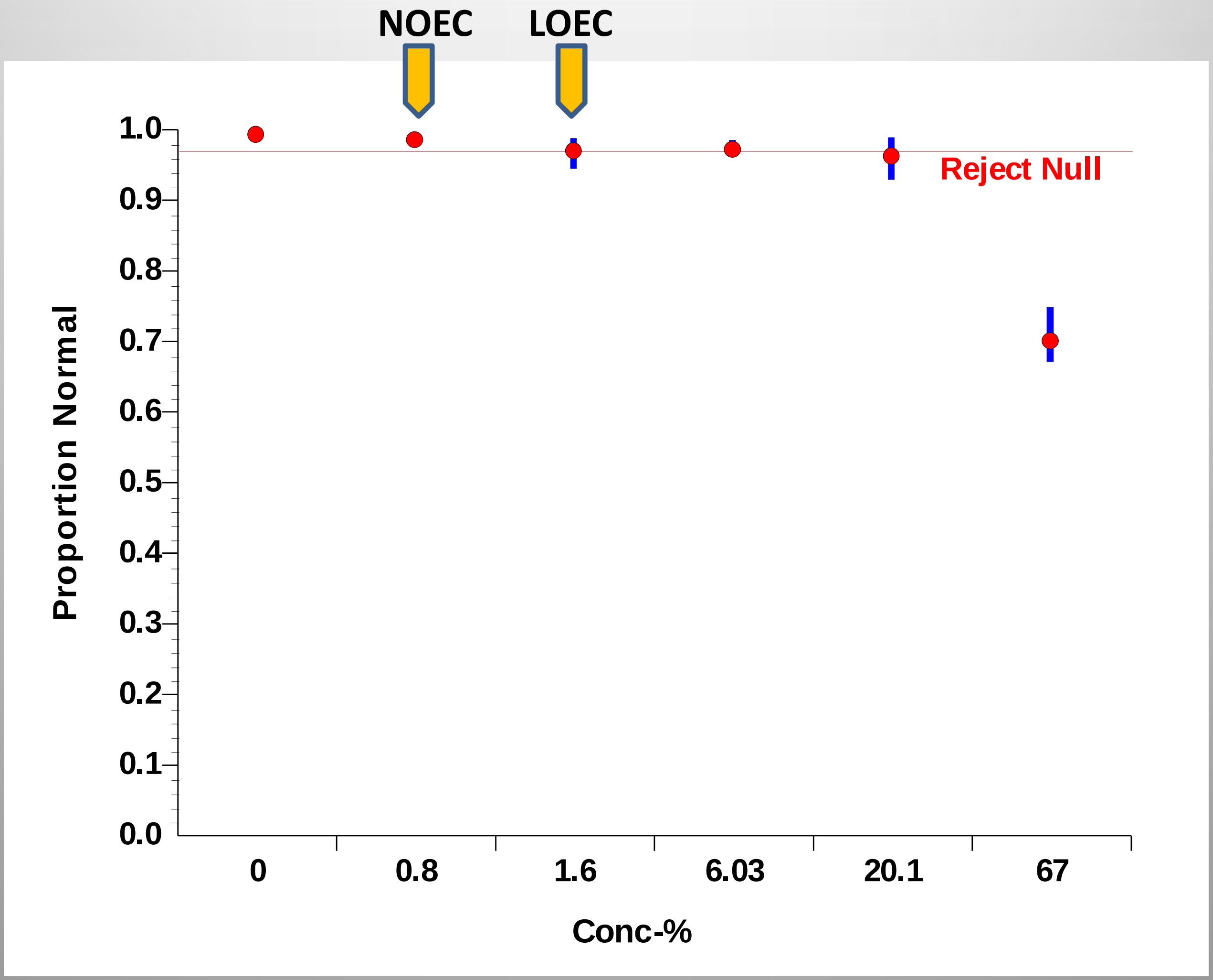


Mean Biomass
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
Reject
0
0.7
1.7
6.25
12.5
25
50
100
Conc-%

Changing *alpha* for small differences

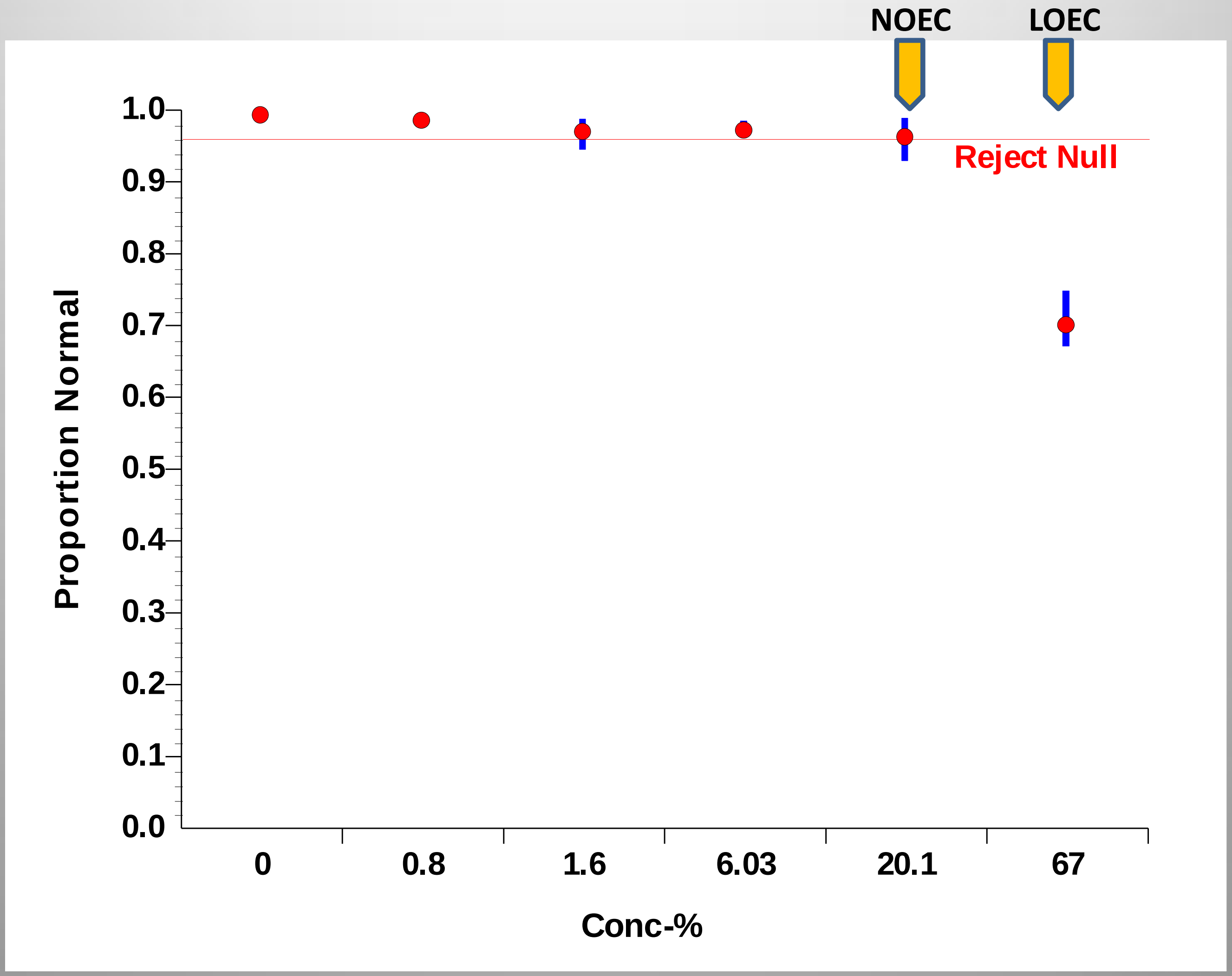
- Change *alpha* from 0.05 to 0.01 when the significant difference in an acute test is less than 10% or the significant difference in a chronic test is less than 20%.
- This minimizes false positives and results meeting anomalous criterion 1.
- Changing *alpha* from 0.05 to 0.01 makes the PMSD bigger.

Alpha = 0.05



NOEC
LOEC
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Proportion Normal
Reject Null
0
0.8
1.6
6.03
20.1
67
Conc-%

Alpha = 0.01



NOEC
LOEC
Reject Null
Proportion Normal
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
0.8
1.6
6.03
20.1
67
Conc-%

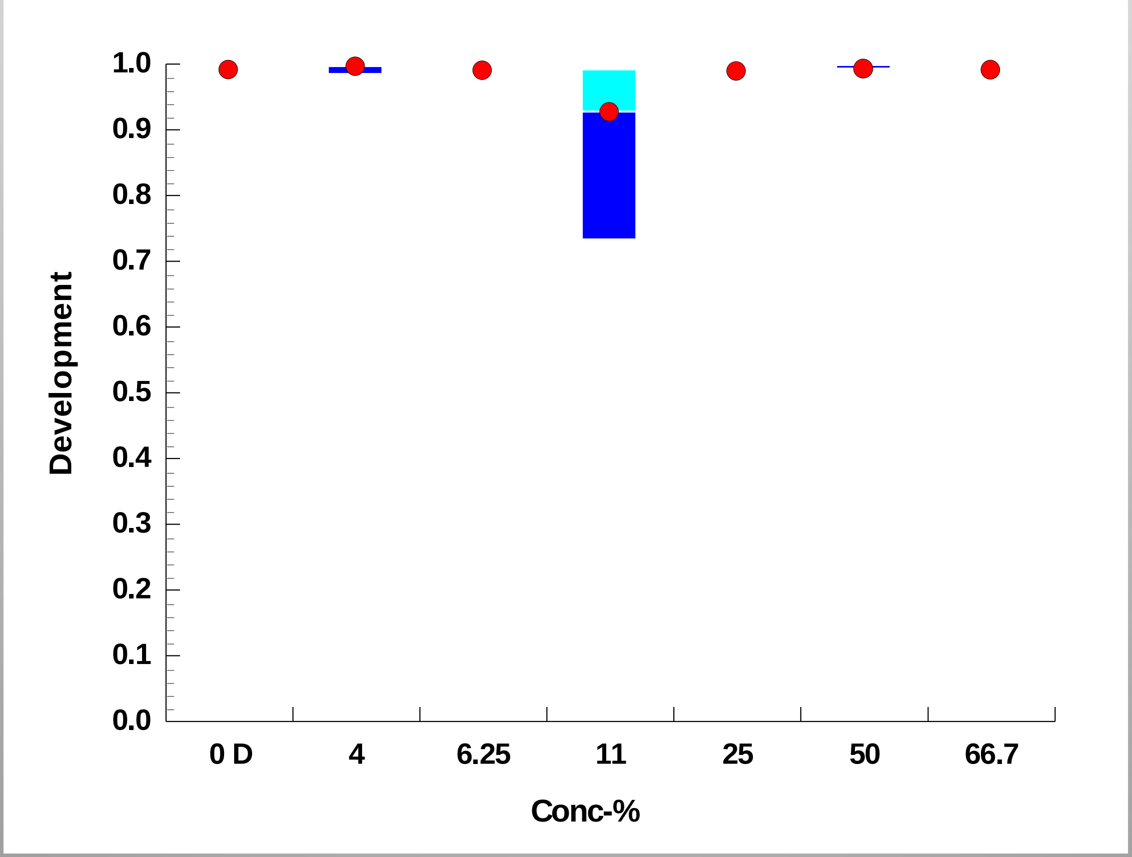
False Positives

- By changing *alpha* to 0.01 for small differences in response, we cap the false positive rate at 1% of tests conducted on nontoxic samples.
- By screening for anomalous concentration-responses, we further reduce false positives.
- By requiring follow-up testing (4 weekly for acute or 3 monthly for chronic) before jumping to conclusions, we avoid unnecessary TI/REs and resolve WET limit noncompliance.
- WAC 173-205-070(1) & (2) base compliance with a limit on the "most recent" test result.

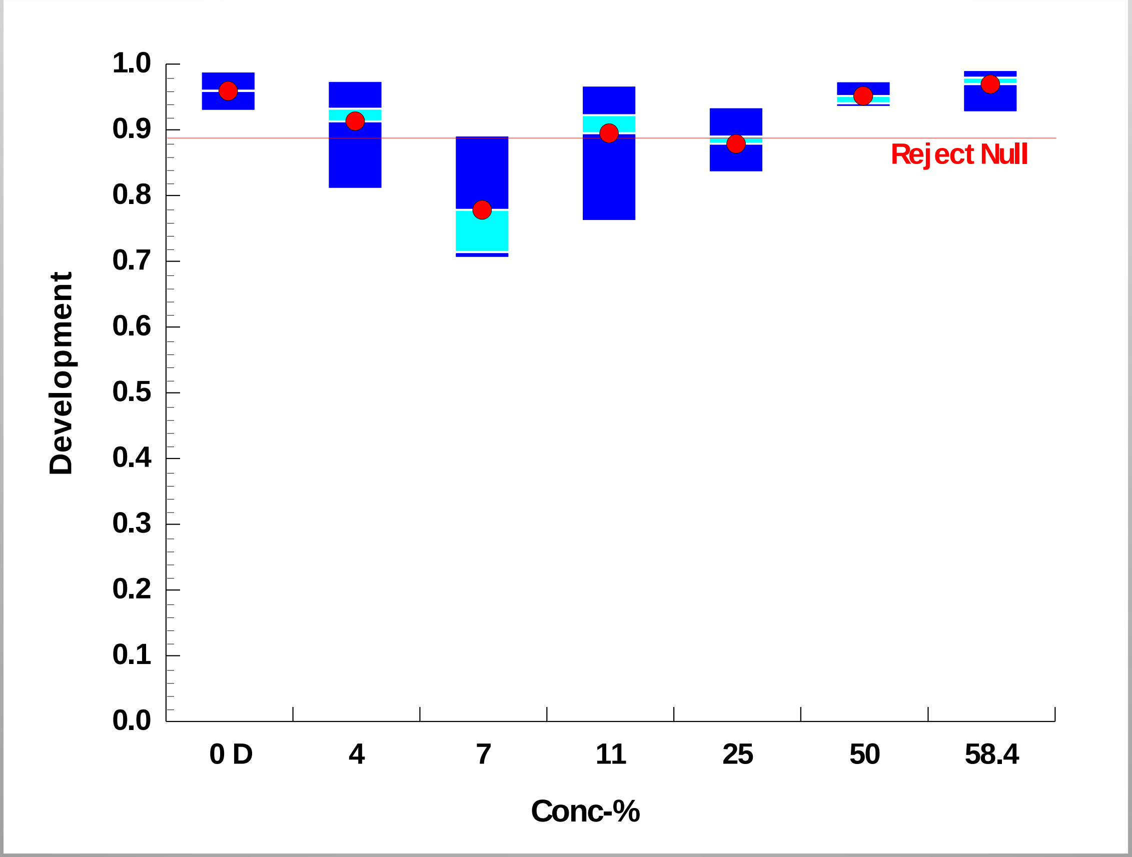
Sometimes it isn’t anomalous.

- Wastewater, stormwater, and receiving water are complex mixtures of chemical constituents and do not always follow the rules for single chemical toxicity.
- The only way to spot atypical concentration-responses that represent toxicity is with historical data. Is the pattern a feature of the discharge?
- The following slides show the interaction of aluminum and fluoride over a year’s monitoring.
- Low concentrations do not have enough aluminum to be toxic and high concentrations have enough fluoride to make aluminum nontoxic. Middle are toxic.

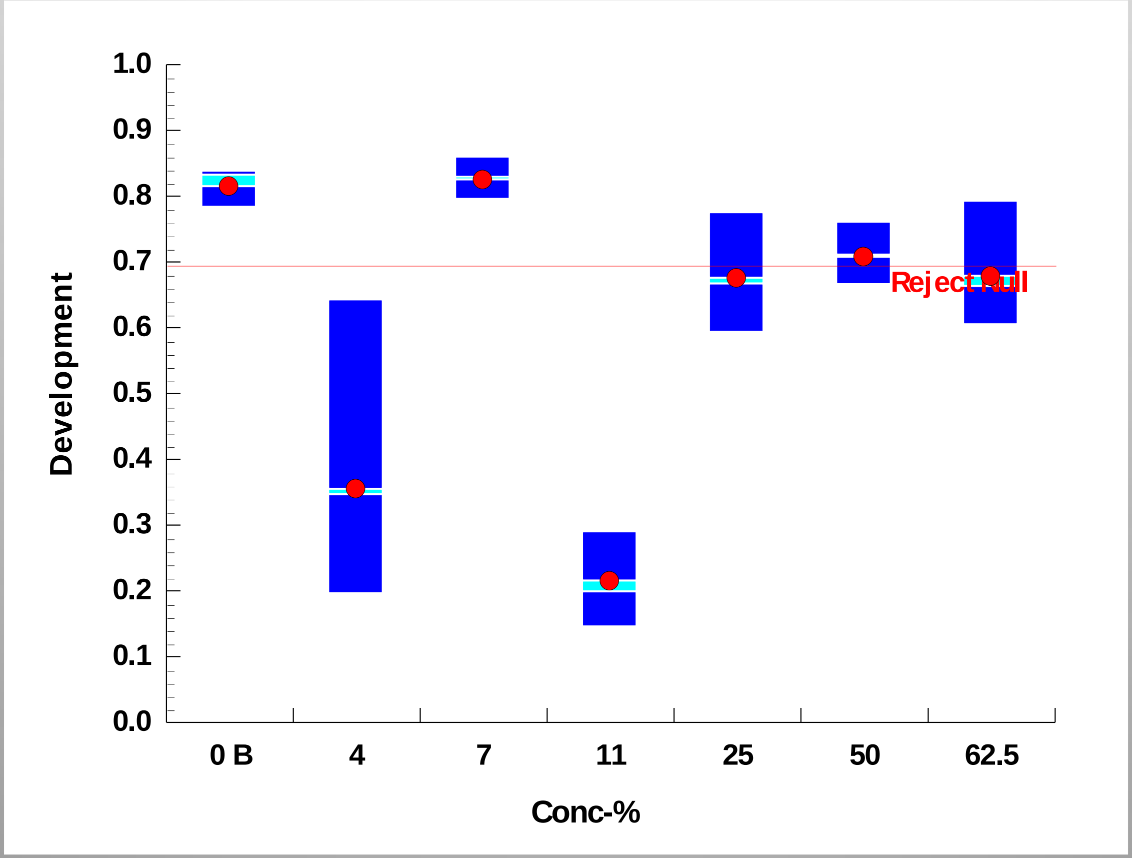
Intalco stormwater 5/24/2000



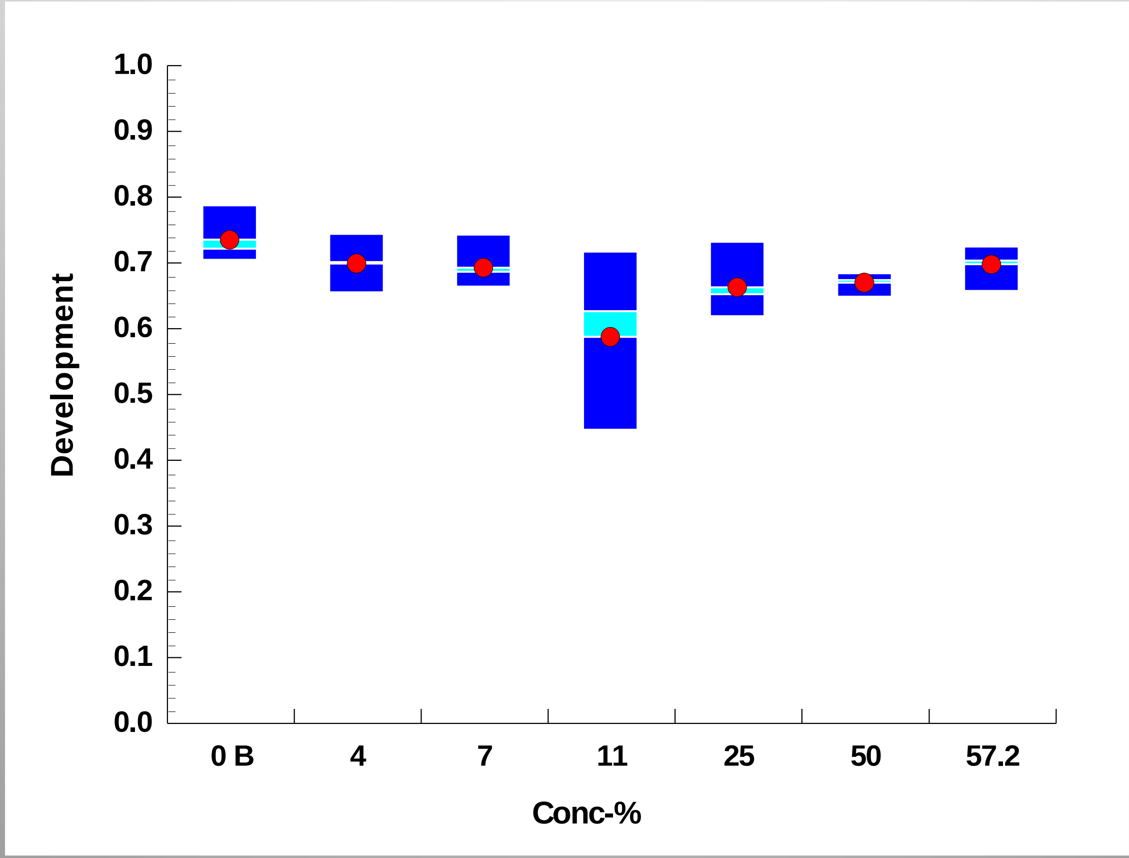
Intalco stormwater 6/27/2000



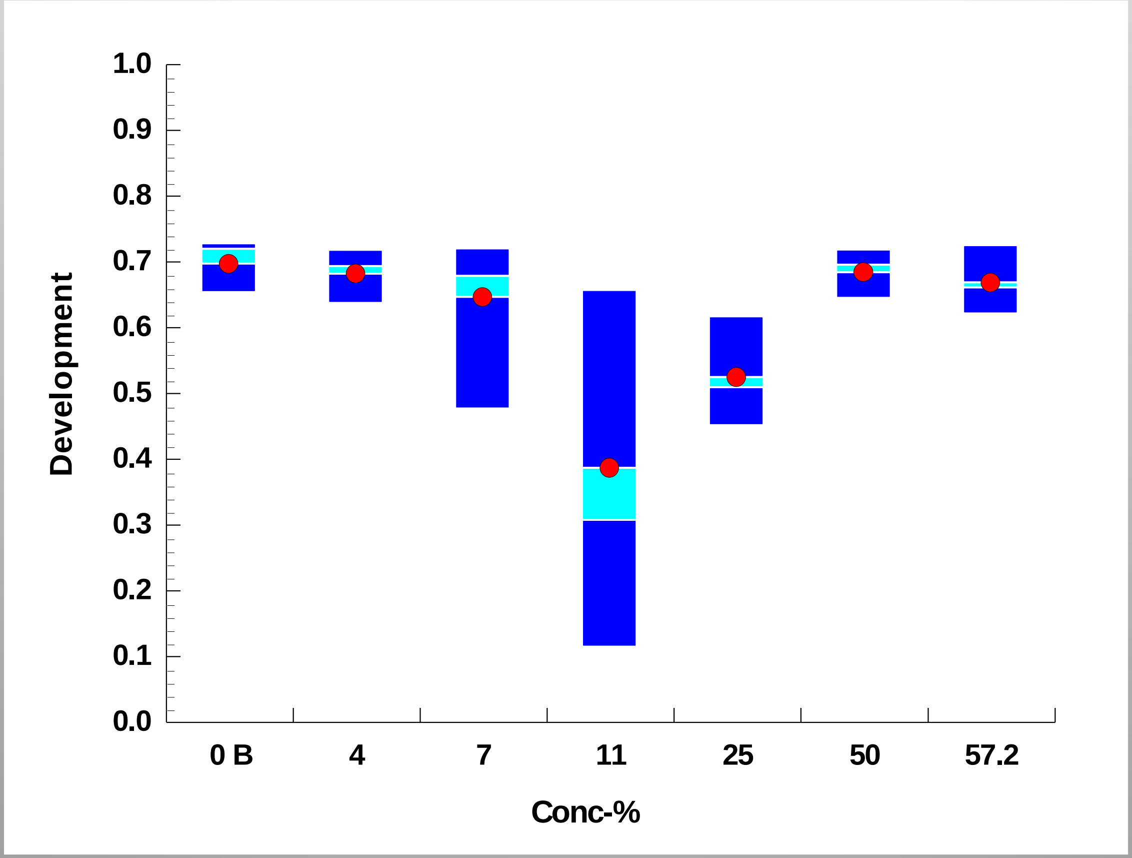
Intalco stormwater 11/7/2000



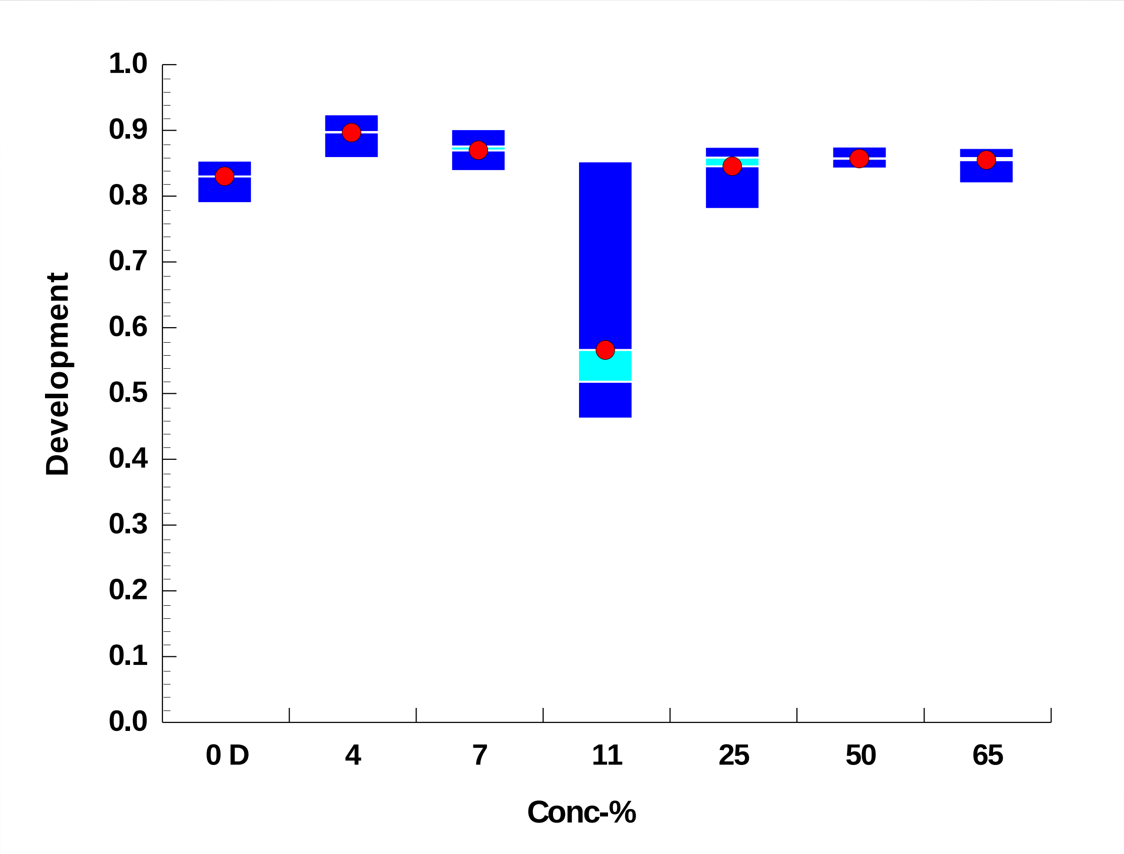
Intalco stormwater 12/20/2000



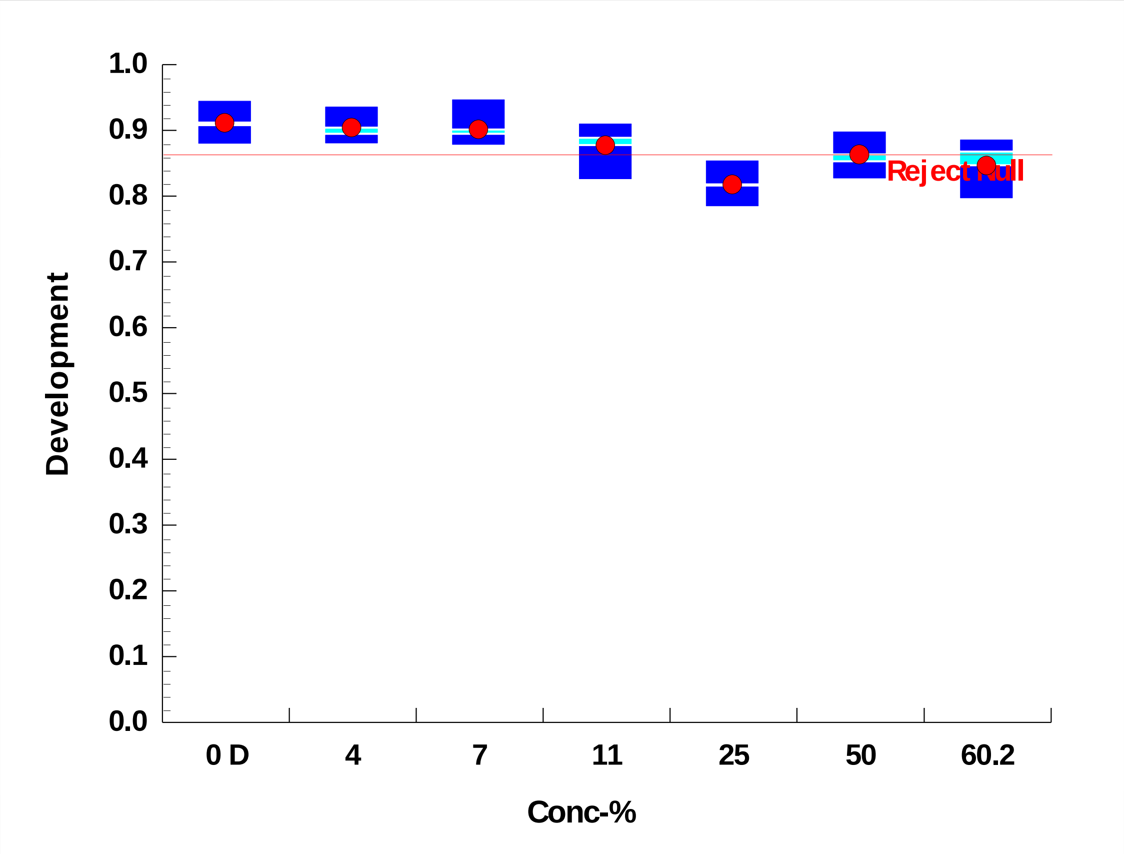
Intalco stormwater 12/20/2000 split



Intalco stormwater 5/15/2001



Intalco stormwater 11/6/2001



Better control of baghouse dust has now reduced toxic response and shifted it to a higher concentration. This was the last sample with significant toxicity.

Reading CETIS reports and Interpreting permittee tables

CETIS Test Evaluation Report

Facility:	Buckhorn Mine	**Test Title:**	Phytoplankton Growth Inhibition Test
Sample Site:		**Organism:**	Pseudokirchneriella subcapitata (Green Alga)
Sample Code:	RMAR1811	**Protocol:**	EPA/821/R-02-013 (2002)
Sample Date:	20 May-09 07:15	**Start Date:**	21 May-09 15:30
Sample Age:	32h (2 °C)	**End Date:**	25 May-09 14:00
Project:		**Test Duration:**	94h **Organism Age:**

Permitee:	Crown Resources Corporation	**Laboratory:**	GEI Consultants / Chadwick
Address:	363 Fish Hatchery Road Republic, WA 99166	**Address:**	4601 DTC Blvd. Suite 900 Denver, CO 80237
Contact:	Kevin Mitchum, Environmental Engineer	**Contact:**	Suzanne Pargee, Laboratory Director
Phone:	(509) 775-3157, x128	**Phone:**	(303) 264-1032
Email:	info@kinross.com	**Email:**	spargee@geiconsultants.com

Chronic Toxicity Evaluation

Endpoint	Parameter	Conc-%	IWC	Pass/Fail	Method
Cell Density	NOEL/LOEL	12.5/25	100	Fail	Steel Many-One Rank Test
Light Absorbance	NOEL/LOEL	12.5/25	100	Fail	Steel Many-One Rank Test

Test Acceptability Criteria

Endpoint	Attribute	Test Stat	Limits	Pass/Fail
Cell Density	Control CV	0.01856	N/A - 0.2	Pass
Cell Density	Control Resp	4400000	1000000 - N/A	Pass
Cell Density	PMSD	0.1013	0.091 - 0.29	Pass

Test Review Comments

This toxicity test is acceptable according to test review criteria. There were no significant deficiencies in sample handling, test performance, test organism response, or statistical analysis.

The test result showed significant chronic toxicity at the ACEC. A chronic WET limit is needed.

Test Reviewer

Reviewer: Randall Marshall, WET Coordinator
Phone: 360-407-6445, 360-407-6426(fax)
Email: rmar461@ecy.wa.gov

Signature Date

CETIS Summary Report

Report Date:	21 Nov-11 14:48 (p 1 of 1)
Test Code:	RMAR2382 | 04-9083-9710

Salmonid Embryo Survival and Development Test — **Nautilus Environmental (Burnaby, BC)**

Batch ID:	13-5797-7681	**Test Type:**	Development-Survival	**Analyst:**	Randall Marshall
Start Date:	21 Sep-11 15:00	**Protocol:**	EC/EPS 1/RM/28	**Diluent:**	Dechlorinated Tap Water
Ending Date:	28 Sep-11 14:00	**Species:**	Oncorhynchus mykiss	**Brine:**	
Duration:	6d 23h	**Source:**	Trout Lodge Fish Farm	**Age:**	

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
05-1168-0731	Development	25	>25	N/A	28.0%	4	Bonferroni Adj t Test
14-1026-5617	Survival	12.5	25	17.68	32.2%	8	Steel Many-One Rank Test

Point Estimate Summary

Analysis ID	Endpoint	Level	%	95% LCL	95% UCL	TU	Method
07-2101-4402	Survival	LC50	12.48	11.58	13.44	8.016	Trimmed Spearman-Kärber

Development Summary

Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	4	0.9917	0.9854	0.9979	0.9667	1	0.008333	0.01667	1.68%	0.0%
6.25		4	0.9917	0.9854	0.9979	0.9667	1	0.008333	0.01667	1.68%	0.0%
12.5		4	0.802	0.6848	0.9191	0.3333	1	0.1569	0.3138	39.13%	19.13%
25		2	0.8333	0.7453	0.9213	0.6667	1	0.1667	0.2357	28.28%	15.97%

Survival Summary

Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Negative Control	4	0.9917	0.9854	0.9979	0.9667	1	0.008333	0.01667	1.68%	0.0%
6.25		4	0.975	0.9563	0.9937	0.9	1	0.025	0.05	5.13%	1.68%
12.5		4	0.4333	0.2685	0.5981	0.06667	1	0.2207	0.4414	101.9%	56.3%
25		4	0.08333	0.04205	0.1246	0	0.2333	0.05528	0.1106	132.7%	91.6%
50		4	0	0	0	0	0	0	0		100.0%
100		4	0	0	0	0	0	0	0		100.0%

CETIS Measurement Report

Report Date:	09 May-12 14:23 (p 1 of 1)
Test Code:	RMAR2501 | 02-4514-8039

Mysid Acute Survival Test — **Nautilus Environmental (San Diego)**

Sample ID:	03-7683-2224	**Code:**	RMAR2501	**Client:**	Bellingham Wastewater Treatment
Sample Date:	13 Mar-12 06:50	**Material:**	POTW Effluent	**Project:**	
Receive Date:	14 Mar-12 09:05	**Source:**	Bellingham WWTP (WA0023744)		
Sample Age:	34h (0.3 °C)	**Station:**			

Sample Measurement	1
Alkalinity (CaCO3)-mg/L	108
Conductivity-µmhos	465
Dissolved Oxygen-mg/L	12
Hardness (CaCO3)-mg/L	70
pH	6.99
Total Ammonia (N)-mg/L	14.2
Total Chlorine-mg/L	0

This page now is included with all summary reports for WET tests. Looking at ammonia can explain some toxicity. Tracking conductivity over time as it relates to toxicity can provide hints. For example, food processing wastewater can be salty or acidic and will raise conductivity. Hardness and alkalinity are discussed on next slide.

Hardness (permanent & temporary) and Alkalinity Constituents

CONSTITUENT	hardness	alkalinity
calcium chloride	P	
calcium sulfate (27.3%)	P	
calcium carbonate	T	X
calcium bicarbonate	T	X
calcium hydroxide		X
magnesium chloride	P	
magnesium sulfate (27.3%)	P	
magnesium carbonate	T	X
magnesium bicarbonate	T	X
magnesium hydroxide		X
sodium carbonate		X
sodium bicarbonate (43.6%)		X
sodium hydroxide		X
potassium carbonate		X
potassium bicarbonate		X
potassium hydroxide		X

If effluent hardness and alkalinity are very different, this table from Appendix J of the Canary Book can be used to get a hint as to why. If hardness is higher, there may be extra chlorides or sulfates. If alkalinity is higher, differences could be due to hydroxides, sodium, or potassium.

Selah WWTP Acute WET Test Results as % Survival in 100% Effluent					
Test Code	Collected	Start Date	Organism	Endpoint	% survival
RMAR0580	4/13/2004	4/14/2004	*Ceriodaphnia dubia*	48-hour Survival	100%
RMAR0579	4/13/2004	4/14/2004	fathead minnow	96-hour Survival	98%
khan087	7/19/2004	7/20/2004	*Ceriodaphnia dubia*	48-hour Survival	100%
khan086	7/19/2004	7/20/2004	fathead minnow	96-hour Survival	85%
khan094	11/16/2004	11/17/2004	*Ceriodaphnia dubia*	48-hour Survival	100%
khan095	11/16/2004	11/17/2004	fathead minnow	96-hour Survival	100%
khan123	1/26/2005	1/27/2005	*Ceriodaphnia dubia*	48-hour Survival	100%
khan125	1/26/2005	1/27/2005	fathead minnow	96-hour Survival	55%
RMAR1877	1/18/2010	1/19/2010	*Ceriodaphnia dubia*	48-hour Survival	100%
RMAR1876	1/18/2010	1/19/2010	fathead minnow	96-hour Survival	98%
RMAR2051	7/19/2010	7/20/2010	*Ceriodaphnia dubia*	48-hour Survival	100%
RMAR2050	7/19/2010	7/20/2010	fathead minnow	96-hour Survival	98%

Results with < 65% survival will require an acute WET limit be put in the permit or prevent the removal of an acute WET limit from the permit.

Selah WWTP Acute WET Test Results as NOEC/LOEC in % Effluent							
Test Code	Collected	Start Date	Organism	Endpoint	NOEC	LOEC	PMSD
RMAR0580	4/13/2004	4/14/2004	*Ceriodaphnia dubia*	48-hour Survival	100	> 100	10.86%
RMAR0579	4/13/2004	4/14/2004	fathead minnow	96-hour Survival	100	> 100	7.46%
khan087	7/19/2004	7/20/2004	*Ceriodaphnia dubia*	48-hour Survival	100	> 100	5.00%
khan086	7/19/2004	7/20/2004	fathead minnow	96-hour Survival	25	50	14.36%
khan094	11/16/2004	11/17/2004	*Ceriodaphnia dubia*	48-hour Survival	100	> 100	5.00%
khan095	11/16/2004	11/17/2004	fathead minnow	96-hour Survival	100	> 100	14.90%
khan123	1/26/2005	1/27/2005	*Ceriodaphnia dubia*	48-hour Survival	100	> 100	5.00%
khan125	1/26/2005	1/27/2005	fathead minnow	96-hour Survival	25	50	15.44%
RMAR1877	1/18/2010	1/19/2010	*Ceriodaphnia dubia*	48-hour Survival	100	> 100	5.00%
RMAR1876	1/18/2010	1/19/2010	fathead minnow	96-hour Survival	100	> 100	5.25%
RMAR2051	7/19/2010	7/20/2010	*Ceriodaphnia dubia*	48-hour Survival	100	> 100	5.00%
RMAR2050	7/19/2010	7/20/2010	fathead minnow	96-hour Survival	100	> 100	5.60%

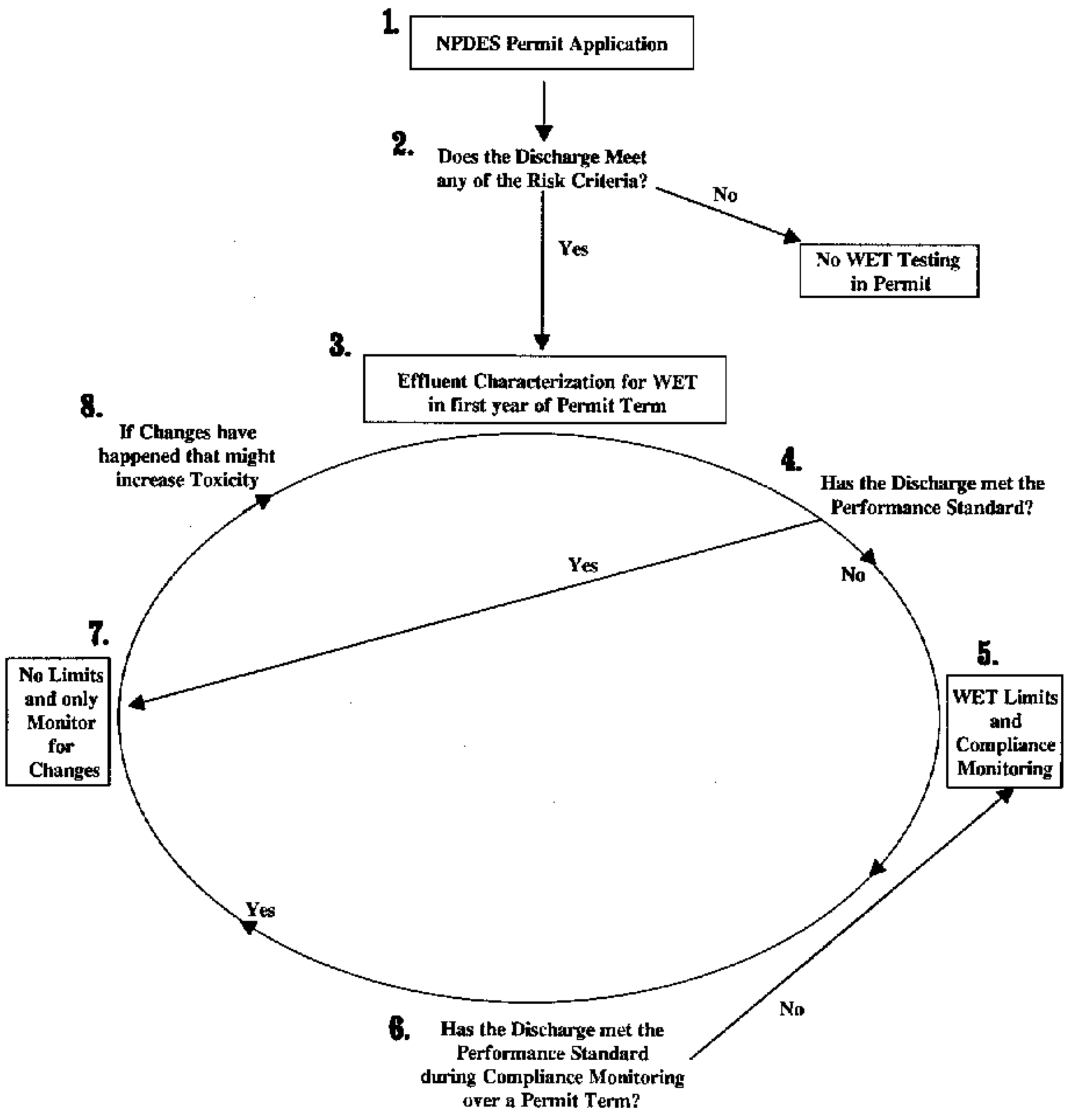
They fail if the LOEC is ≤ the ACEC . If the NOEC is ≥ the ACEC, they pass. All tests met the acute power standard because PMSDs were < 30%

Prosser WWTP Chronic WET Test Results as NOEC/LOEC in % Effluent

Test Code	Collected	Start Date	Lab	Organism	Endpoint	NOEC	LOEC	PMSD
khan092	9/22/2004	9/23/2004	Nautilus Environmental	*Ceriodaphnia dubia*	7-day Survival	100	> 100	
					Reproduction	56	100	24.75%
khan093	9/22/2004	9/23/2004	Nautilus Environmental	fathead minnow	7-day Survival	100	> 100	8.26%
					Biomass	100	> 100	15.53%
					Weight	100	> 100	14.43%
khan096	9/29/2004	9/30/2004	Nautilus Environmental	green alga	Cell Density	100	> 100	7.51%
khan131	9/26/2005	9/27/2005	Nautilus Environmental	*Ceriodaphnia dubia*	7-day Survival	100	> 100	
					Reproduction	100	> 100	41.22%
		/2005	Nautilus Environmental	fathead minnow	7-day Survival	100	> 100	2.50%
					Biomass	100	> 100	14.20%
					Weight	100	> 100	14.20%
		/2005	Nautilus Environmental	green alga	Cell Density	12.5	25	12.17%
		/2007	Nautilus Environmental	fathead minnow	7-day Survival	100	> 100	9.98%
					Biomass	100	> 100	14.27%
					Weight	100	> 100	14.39%
		/2007	Nautilus Environmental	*Ceriodaphnia dubia*	7-day Survival	100	> 100	
					Reproduction	100	> 100	34.44%
RMAR1254	4/30/2008	5/1/2008	Nautilus Environmental	green alga	Cell Density	100	> 100	13.13%
RMAR1361	11/10/2008	11/11/2008	Nautilus Environmental	fathead minnow	7-day Survival	100	> 100	5.60%
					Biomass	100	> 100	12.22%
					Weight	100	> 100	10.89%
RMAR1419	1/5/2009	1/6/2009	Nautilus Environmental	fathead minnow	7-day Survival	100	> 100	28.76%
					Biomass	100	> 100	27.03%
					Weight	100	> 100	22.79%
RMAR1596	5/4/2009	5/5/2009	Nautilus Environmental	*Ceriodaphnia dubia*	7-day Survival	100	> 100	
					Reproduction	50	100	42.20%
RMAR1806	11/12/2009	11/13/2009	Nautilus Environmental	green alga	Cell Density	101	> 100	7.88%
RMAR2004	5/24/2010	5/25/2010	Nautilus Environmental	fathead minnow	7-day Survival	100	> 100	7.15%
					Biomass	100	> 100	20.04%
					Weight	100	> 100	23.14%
RMAR2167	12/6/2010	12/7/2010	Nautilus Environmental	*Ceriodaphnia dubia*	7-day Survival	100	> 100	
					Reproduction	100	> 100	30.03%

They fail if the LOEC is ≤ the CCEC . If the NOEC is ≥ the CCEC, they pass. If the PMSD is > 40%, the test did not meet the chronic power standard.

WET PERMITTING SYSTEM DIAGRAM



permitting steps 1 - 4

Step 1 - The process begins with a National Pollutant Discharge Elimination System (NPDES) permit application.

Step 2 - Section 173-205-040 of the WET rule contains a list of circumstances under which a discharge is required to be characterized for WET.

Step 3 - WET testing usually begins with an effluent characterization in the first year of the permit term. Effluent characterization establishes the baseline toxicity level and determines the need for WET limits. Every sample during effluent characterization will be tested with all of the WET tests listed in the permit (multiple species testing).

Step 4 - The performance standard for acute toxicity is no test result < 65% survival in 100% effluent. The performance standard for chronic toxicity is no chronic toxicity in a concentration representing the edge of the acute mixing zone. Those permittees who meet the performance standards will not get WET limits or compliance monitoring (will go straight to Step 7).

permitting steps 5 - 8

Step 5 - Those permittees who do not meet a performance standard during effluent characterization will receive WET limits.

Step 6 - If a permittee with a WET limit meets the performance standard for an entire permit term, then the WET limit will not be placed into subsequent permits.

Step 7 - Permittees who have attained the performance standards can remain indefinitely without WET limits or compliance monitoring. The only WET testing requirement will be one set of WET tests submitted with each permit application. Some permittees will be required to conduct rapid screening testing. All facility changes must be evaluated for increases in toxicity.

Step 8 - If changes have occurred which might increase toxicity, then the next permit will contain a requirement for a new effluent characterization. The new effluent characterization will start the process all over again beginning at Step 3.

Occurrence of acute toxicity

- Since the performance standard for acute toxicity is based upon % survival in 100% effluent, it is easy to see the failure rate.
- 26% of industrial stormwater samples had < 65% survival.
- 23% of oil refinery samples had < 65% survival.
- 18% of POTW samples had < 65% survival.
- All other large categories did much better.

Ammonia explains a lot.

- 39% of effluent samples with < 65% survival came from POTWs.
- Most, if not all, of POTW acute toxicity can be explained by ammonia.
- Effluent ammonia levels ≥ 12 mg/L might be acutely toxic to fish depending on pH.
- Effluent ammonia levels ≥ 25 mg/L might be acutely toxic to daphnids depending on pH.

Phase I trout embryo testing

- Transportation and associated infrastructure are major sources of copper and zinc.
- Seasonal first flush metals concentrations were more pronounced for commercial landuse (3.5 to 4 times above average) rather than residential (1.5 to 2.5 times higher).
- Toxicity was only seen in commercial landuse areas (including Ports) and tracked copper and zinc concentrations.

Acute WET is uncommon, not changing, and usually no threat.

- 83% of tests had at least 65% survival in 100% effluent from 1990 to 1997.
- 83% of tests had at least 65% survival in 100% effluent from 1997 to 2009.
- 2:1 dilution eliminated statistically significant mortality in 57% of tests with toxicity.
- 4:1 dilution eliminated statistically significant mortality in 81% of tests with toxicity.

Chronic WET is uncommon, not changing, and usually no threat.

- 59% of NOECs equaled 100% effluent from 1990 to 1997.
- 60% of NOECs equaled 100% effluent from 1997 to 2009.
- 2:1 dilution eliminated statistically significant adverse effects in 35% of tests with toxicity.
- 4:1 dilution eliminated statistically significant adverse effects in 66% of tests with toxicity.

Monitoring Frequency

- The 17% of acute tests with < 65% survival in 100% effluent came from 52% of permittees.
- A discharge toxic 10% of the time has a 66% chance of passing all quarterly tests in a year and a 28% chance of passing all monthly testing.
- 26 samples per year minimum would be needed to have a 95% chance of catching at least 1 toxic episode from a discharge toxic 10% of the time.
- Toxicity is either on or off depending on whether a pollutant is above or below its toxic threshold.

Recommendations and Advice

- Don’t require WET testing unless mixing zone and dilution factor determinations are complete.
- Always include the ACEC and/or CCEC in a concentration series.
- If you already know that it is toxic, why test? For example, parking lot runoff often contains toxic concentrations of copper and zinc and knowing this is enough to begin fixing the problem.
- The WQS and EPA’s EcoTox database are free to use and can tell us a lot about the toxicity of the most common pollutants.

Recommendations and Advice, cont.

- WET tests are best for finding unknown toxicants.
- Unknown toxicants must become known toxicants before they can be fixed.
- Don't require toxicity testing without a process for fixing toxicity. If you cannot define a process, maybe toxicity testing is not appropriate for the situation.
- WET tests are merely tools for finding and fixing effluent toxicity. They are run in a lab at a temperature, hardness, food quality and quantity, etc. very different from the receiving environment.

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